Online Supplemental Material

Apolipoprotein M and sphingosine-1-phosphate receptor 1 promote the transendothelial transport of HDL

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Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse (*Mus Musculus*) C57Bl/6J	Charles River Laboratories, Sulzfeld, Germany	C57Bl/6J	F	https://www.jax.org/strain/000664

Genetically Modified Animals

	Species	Vendor or Source	Back-ground Strain	Other Infor-mation	Persistent ID / URL
Parent - Male	Mouse (*Mus Musculus*) Tg(Cdh5-cre/ERT2)CIVE23Mlia X B6.129P2-Apoetm1Unc/J (referred to as *Apoe$^{-/-}$ Cdh5-CreER^{T2}*)	Christian Weber (Ludwig Maximillian University, München, Germany)	C57Bl/6J		*http://www.informatics.jax.org/allele/MGI:3700149* *https://www.jax.org/strain/002052*
Parent - Female	Mouse (*Mus Musculus*) *C57Bl/6J-Gt(ROSA)26Sor$^{tm1(S1pr1)Geno}$ (referred to as S1pr1 ^{LSL}* line)	Genoway, Lyon, France	C57Bl/6J	Generated on behalf of University of Modena and Reggio Emilia, Italy	Not available

Cultured Cells

Name	Vendor or Source	Sex	Catalogue #
Bovine aortic endothelial cells (BAECs).	Veal calf aorta, Slaughterhouse Zurich	Unknown	Not applicable
Human aortic endothelial cells (HAECs)	Cell Applications Inc	Male and female	304-05a

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration (µg/mL)
$S1P_1$, Western blot	Abcam	ab125074	0.3
$S1P_1$, immunofluorescence	MyBiosource	MBS8534430	20
SR-BI , Western blot	Novus	NB400-131	3.0
SR-BI, immunofluorescence	Novus	NB400-101	20
LDL-receptor, Western blot	Abcam	ab52818	0.7
Na+/K+- ATPase, Western blot	Santa Cruz	SC-21712	1.0
TATA binding protein, Western blot	Abcam	ab51841	1.0
VE-cadherin, Immunofluorescence	Novus	NBP1-43347	10
Goat Anti-Rabbit Immunoglobulins /HRP	Agilent Dako	P0448	0.1
Rabbit Anti-Goat Immunoglobulins/ HRP	Agilent Dako	P0449	0.2
Rabbit Anti-Mouse Immunoglobulins/ HRP	Agilent Dako	P0260	0.5
Secondary antibodies Immunofluorescence	Santa Cruz	sc-516250 sc-516248	1,3
Secondary antibodies Immunofluorescence	Novus	NBP1-76096 NBP1-72973	3.3

Small Interfering RNAs

Gene	Vendor or Source	Catalogue #	Final concentration
$S1P_1$	Ambion silencer select	s4449, s4447 s4448	5nmol/L
SCARB1	Ambion silencer select	s2648 s2649 s2650	5nmol/L
Non-silencing control siRNA	Ambion silencer select	4390843	5nmol/L

Primer sequences

Primer name	Sequence
S1pr1^{LSL} excised allele - forward	AAC ATA CTC CCT TCC CGC AGA AACC
S1pr1^{LSL} excised allele - reverse	AGC CTC TGC TAA CCA TGT TCA TGCC
mS1pr1 – forward	GCA ACT TAA TTT TAT TAG GAC AAG GCT GGT GG
mS1pr1 - reverse	TCT TCA TCC TAC TAC TGT TAG ATG TGG GCT GC
WT *Rosa26* Locus - forward	CAA TAC CTT TCT GGG AGT TCT CTGC
WT *Rosa26* Locus - reverse	CTG CAT AAA ACC CCA GAT GAC TACC
VeCAD_Cre - forward	ATC CGA AAA GAA AAC GTT GA
VeCAD_Cre - reverse	ATC CAG GTT ACG GAT ATA GT
hS1PR1 - forward	GTC TGG AGT AGCGCC ACC
hS1PR1 - reverse	GTA GTC AGA GAC CGA GCT GC
hSCARB1 - forward	CTG TGG GTG AGA TCA TGT GG
hSCARB1 – reverse	GCC AGA AGT CAA CCT TGC TC
hGAPDH – forward	CCC ATG TTC GTC ATG GGT GT
hGAPDH - reverse	TGG TCA TGA GTC CTT CCA CGA TA

Supplemental table I: Treatment with S1P1 agonist SEW2871 differentially regulates endothelial permeability for HDL and Evan's Blue in wild type mice

	vehicle	SEW 2871	P*
Evans' Blue in the peritoneal fluid (arbU)	0.32 ± 0.02 (N = 5)	0.26 ± 0.01 * (N = 5)	0.041
DyL-HDL in the peritoneal fluid (arbU)	78.2 ± 5.2 (N = 8)	106.8 ± 13.2* (N = 8)	0.032

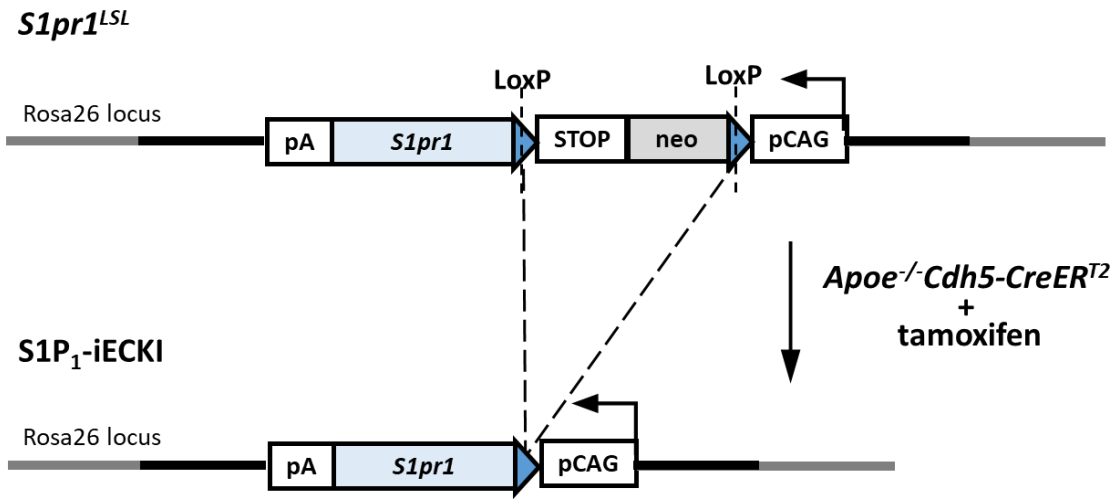
i.v. injection of Evan's Blue (600 µg/animal) or DyLight-HDL (350µg/animal) and i. p. stimulation with lipopolysaccharide (25.0 µg/animal). SEW2871 administration (15µg/g body weight) 150 min prior to stimulation with LPS. Collection of peritoneal fluid 3h after stimulation with LPS. (Results are presented as means ± SEM; *: P calculated by Mann-Whitney U-Test. (arbU = arbitrary units)

Supplemental Table II: Less severe hypercholesterolemia and fatty streak formation in S1P1-iECKI mice fed with a high fat diet

	CTRL (N = 5)	$S1P_1$-iECKI (N = 5)	P*
Total cholesterol (mmol/L)	6.56 ± 0.25	4.66 ± 0.27	< 0.001
HDL-cholesterol (mmol/L)	2.21 ± 0.16	1.57 ± 0.10	0.0086
nonHDL cholesterol (mmol/L)	4.34 ± 0.17	3.10 ± 0.35	0.0127
Triglycerides (mmol/L)	0.41 ± 0.02	0.41 ± 0.04	0.970
Oil red stained area in the aortic roots (% plaque/total area)	1.38 ± 0.24	0.35 ± 0.11	0.0087

Apoe haploinsufficient mice with ($S1P_1$-iECKI) or without (CTRL) a knock-in of *S1p1* were fed for 30 weeks with a western diet containing 1.25% cholesterol before they were sacrificed for collection of blood and aortas. (Results are presented as means ± SEM; *: P calculated by Mann-Whitney U-Test).

a



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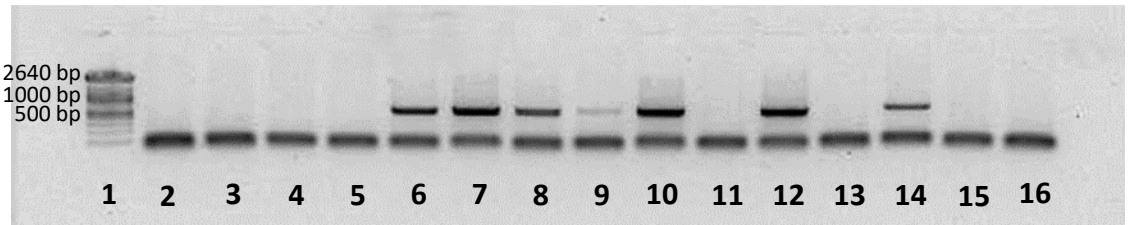
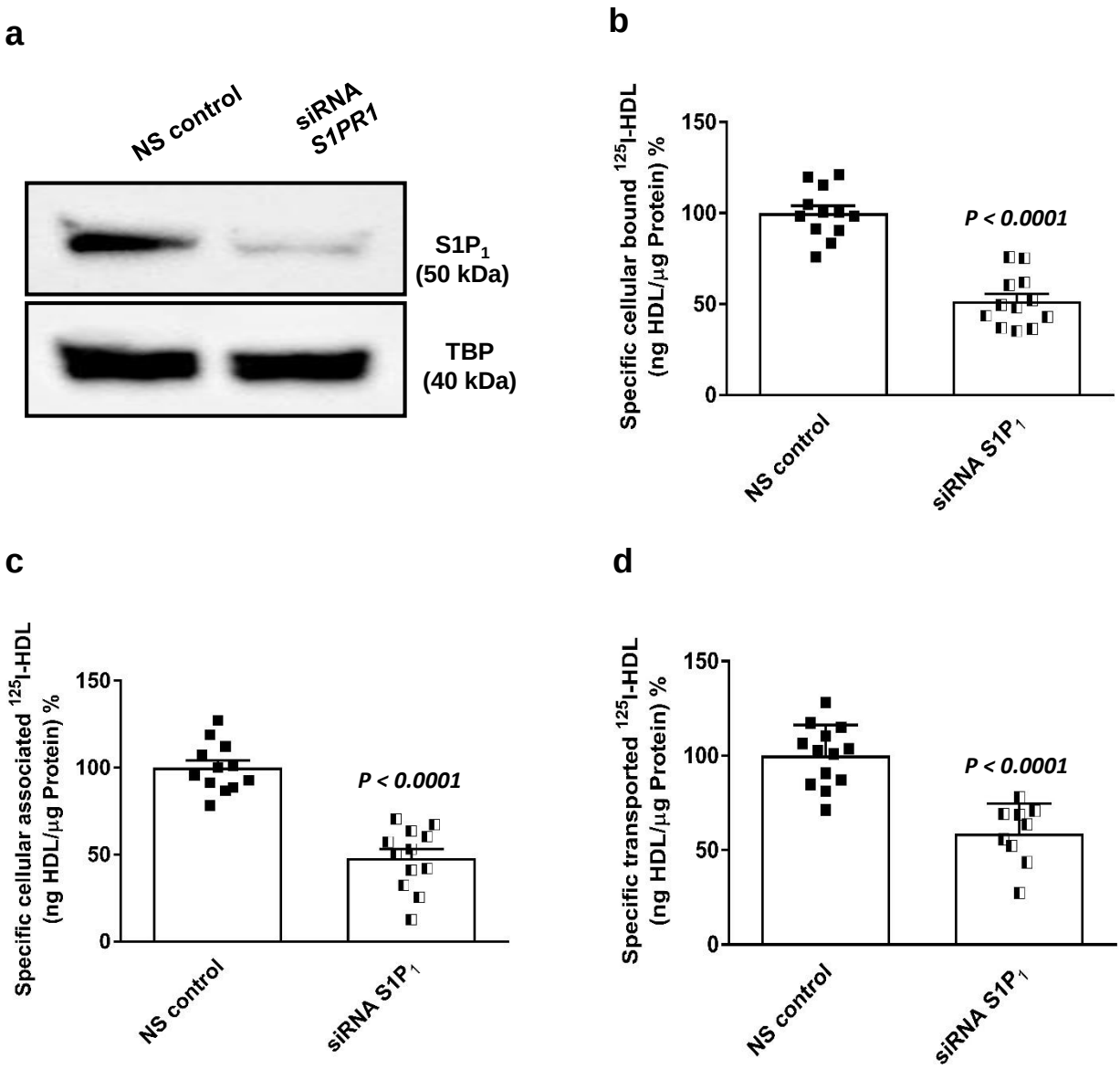
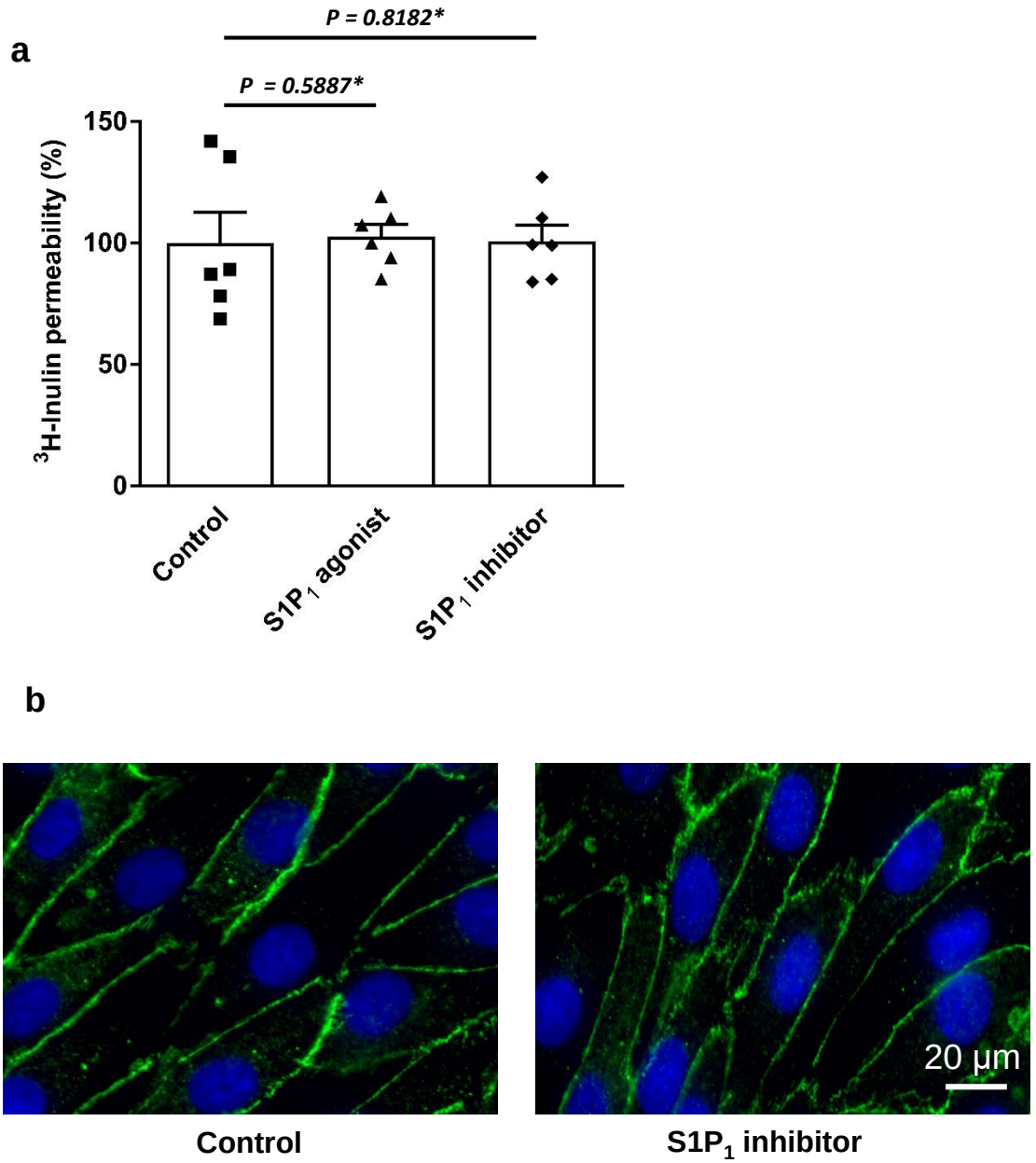


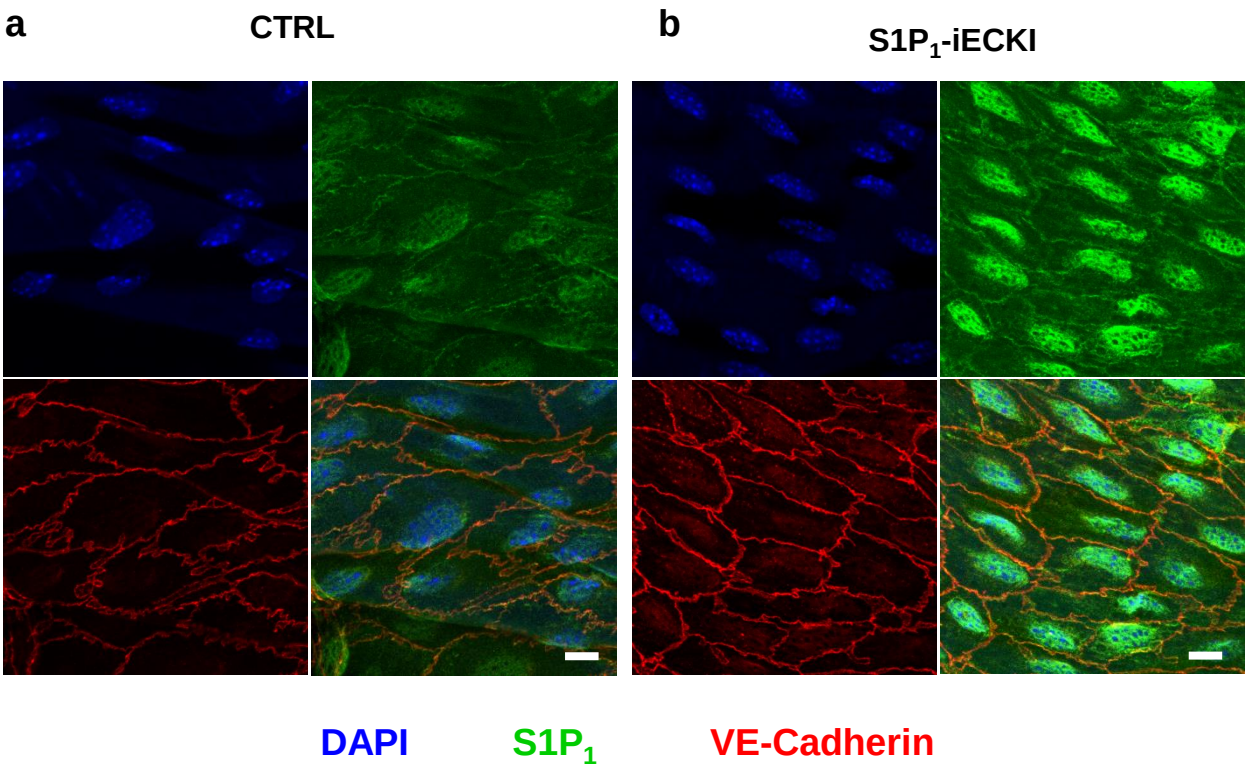
Supplementary Figure I: Targeting vector/transgene structure and Cre-mediated activation of transcription. (**a**) The transgene, inserted within the ROSA26 locus via homologous recombination, contains the murine *S1pr1* cDNA, separated from the synthetic cytomegalovirus early enhancer/chicken β-actin (CAG) strong promoter by a LoxP-STOP-Neomicin-LoxP (LSL) cassette. *S1pr1*^{LSL} mice were crossed with the tamoxifen inducible *Apoe*$^{-/-}$*Cdh5-CreER*^{T2} mice, which express the Cre recombinase under control of the VE-cadherin promoter, active in endothelial cells only. Gene overexpression was achieved by intraperitoneal injection of tamoxifen and the *LSL* insert is hence excised only in Cre-expressing cells. $S1P_1$-iECKI: $S1P_1$- inducible endothelial cell knock-in mice. **(b)** Agarose gel electrophoresis of PCR-amplified genomic DNA from lungs of wild type mice and S1P1-ECKI mice. From the left: lane 1 molecular weight marker (Marker XIV, Roche); lanes 2 through 15, fourteen samples of mouse genomic DNA; lane 16 water (last lane). Upper bands (≈600bp) in samples depicted in lanes 6, 7, 8, 9, 10, 12, and 14 identify samples of S1P1 ECKI mice with the excision event . The lower band is generated by the primers.



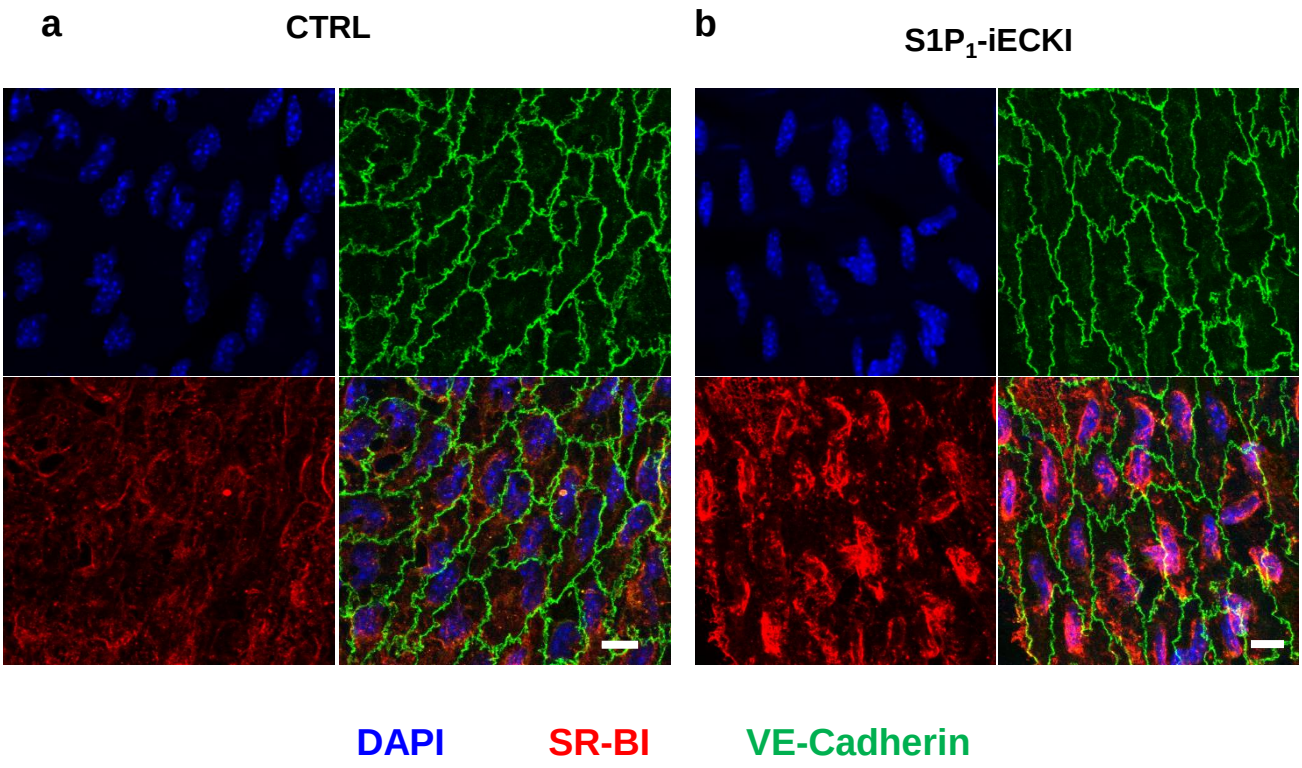
Supplementary Figure II: RNA interference with $S1P_1$ inhibits binding, association, and transport of HDL by human aortic endothelial cells (HAECs). HAECs were transfected with a specific siRNA against $S1P_1$ with non-silencing control siRNA (NS control) and functional assays were performed 72 hours post-transfection. (**a**) representative Western blot showing the efficacy of $S1P_1$ silencing relative to the non-silencing siRNA (NS control) and TATA-binding protein (TBP) used as the loading control. HAECs were incubated with 10μg/mL of ^{125}I-HDL for 1 hour in the absence (total) or in the presence of 40-fold excess of unlabeled HDL, to record nonspecific interactions. Specific binding, association and transport were calculated by subtracting nonspecific values from total values. (**b**) cellular binding of ^{125}I-HDL at 4 ºC. (**c**) cellular association of ^{125}I-HDL at 37 ºC. (**d**) for the measurement of transport of ^{125}I-HDL from the apical to the basolateral compartment, HAECs were cultured on inserts. Data represented as means ± SEM of three or four independent triplicate experiments (n=3). P was calculated by unpaired Mann Whitney U-test



Supplementary Figure III: Endothelial barrier function in the presence of S1P1 receptor inhibitor (a) shows the filtration of tritiated inulin through HAECs cultivated in a transwell system: confluent cells were incubated for 30 minutes at 37 ºC with 20nM of the $S1P_1$ agonist SEW2871 or 20nM of the $S1P_1$ inhibitor W146. **(b)** shows the immunostaining of endothelial tight junction protein (TJP-1) in cultivated HAECs treated with either 20nM of the $S1P_1$ inhibitor W146 or control medium .



Supplementary Figure IV: Demonstration of S1P1 in the endothelium of aortas from Apoe haploinsufficient mice without (a; CTRL) or with overexpression of S1P1 (b, S1P1-iECKI). Figure shows en-face prepared aortic immunostainings. Aortas were quickly cleared from the adventitial tissue, opened longitudinally and incubated with primary and secondary antibodies conjugated with green or red fluorescent dyes, as indicated. Nuclei were counterstained with DAPI. Images were captured by confocal microscope and z-axis projections of 14 scanned planes are shown. Scale bar = 10µm.



Supplemental Figure V: Demonstration of SR-BI in the endothelium of aortas from Apoe haploinsuffS5Vicient mice without (a; CTRL) or with overexpression of S1P1 (b, S1P1-iECKI). Figure shows en-face prepared aortic immunostainings. Aortas were quickly cleared from the adventitial tissue, opened longitudinally and incubated with primary and secondary antibodies conjugated with green or red fluorescent dyes, as indicated. Nuclei were counterstained with DAPI. Images were captured by confocal microscope and z-axis projections of 14 scanned planes are shown. Scale bar = 10µm.